Endogenous IL-13 Limits Humoral Responses and Injury in Experimental Glomerulonephritis but Does Not Regulate Th1 Cell-Mediated Crescentic Glomerulonephritis

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IL-13 is produced by T helper 2 (Th2) cells, has a role in stimulating Th2-mediated injury, alters humoral responses, and may directly suppress macrophage and neutrophil function. In immune renal disease, the engagement of different effector mediator systems, including humoral and cell-mediated effectors, can result in glomerular injury. Experimental crescentic glomerulonephritis (known as autologous anti–glomerular basement membrane glomerulonephritis) induced by planting an antigen in glomeruli of mice is Th1 directed, delayed-type hypersensitivity (DTH)-like, and antibody independent. To test the hypothesis that, like the counterregulatory Th2 cytokines IL-4 and IL-10, endogenous IL-13 limits effector Th1 responses in glomerulonephritis, crescentic glomerulonephritis was induced in IL-13+/- mice and IL-13−/− mice. Although IL-13−/− mice developed increased serum antigen–specific antibody levels, increased glomerular antibody deposition and enhanced switching to the Th1-associated IgG2a subclass, they developed a similar degree of crescentic glomerulonephritis, with similar glomerular T cell/macrophage numbers, renal impairment, and proteinuria. Antigen-specific dermal DTH and IFN-γ production by antigen-stimulated splenocytes was unaltered. In immune complex (apoeritinin-induced) glomerulonephritis, where renal injury is humorally mediated, IL-13−/− mice developed enhanced humoral immune responses and increased proteinuria, with increased IgG2a responses, a more peripheral distribution of immune complexes, but no alternations in leukocyte recruitment. These results demonstrate dissociation of IL-13’s effects in antigen induced renal disease with little effect on cellular responses but suppressive effects on humoral effectors and switching to IgG2a. They indicate a role for IL-13 in limiting antibody-mediated renal injury, but not in regulating DTH-like cell-mediated responses in the kidney.

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ular injury results in the proliferation of cells within the urinary space. This lesion has a number of similarities to a Th1-directed DTH-like response (11). Previous studies have defined experimental crescentic GN as a Th1-directed DTH-like lesion negatively regulated by endogenous IL-4 and IL-10 (13–16). This model is known as accelerated autologous anti-glomerular basement membrane (GBM) GN. Planting an exogenous antigen (sheep globulin [SG]) in glomeruli of sensitized mice induces GN. Severe injury is effector CD4+ dependent (13) but antibody independent (17). Crescent formation is mediated by IL-12 (14), GM-CSF (18), and IFN-γ (19) and is limited by endogenous IL-4 and IL-10 (15,16). Circulating immune complexes are formed by the interaction of soluble antigen with antibody, often IgG. These humoral mediators of injury may be deposited in the glomerulus and cause injury by virtue of their size and charge, and their ability to recruit and effectors such as leukocytes and complement. Immune complexes are important in the pathogenesis of number of renal diseases, including lupus nephritis, postinfectious GN, and serum sickness (20).

The study presented here sought to define the role of endogenous IL-13 in antigen induced immune renal injury by studying mice genetically deficient in IL-13 (3). As endogenous production of other Th2 cytokines (IL-4 and IL-10) limit disease, the hypothesis that IL-13 would limit experimental Th1-directed DTH-like crescentic GN was tested by studying disease and immune responses in IL-13−/− mice. The hypothesis that IL-13 would alter humoral immune responses by altering Ig production and affecting IgG subclass switching was tested by assessing humoral immune responses in the autologous anti-GBM GN model, and in addition by studying GN in apoferritin-induced immune complex GN, a model of immune renal injury mediated primarily by adaptive humoral responses. Last, the potential of IL-13 to contribute to renal fibrosis was assessed in a more chronic phase of Th1-directed glomerular injury.

Materials and Methods

Experimental Design

IL-13−/− mice (C57BL/6 × 129/J background) and IL-13+/+ mice (on the same background) were created as described previously (3), obtained from Dr. Andrew McKenzie (MRC Laboratory of Molecular Biology, Cambridge, United Kingdom) and bred at Monash University, Victoria, Australia. IL-13−/− mice were generated by intercrossing IL-13−/− and IL-13+/+ mice. The genotype of IL-13+/+, IL-13−/−, and IL-13−/− mice was confirmed by a PCR-based protocol. Each identical sample was amplified twice, with one set of primers detecting the disrupted allele via the neomycin insert based protocol. Each identical sample was amplified twice, with one set of primers detecting the disrupted allele via the neomycin insert based protocol. Each identical sample was amplified twice, with one set of primers detecting the disrupted allele via the neomycin insert based protocol. Each identical sample was amplified twice, with one set of primers detecting the disrupted allele via the neomycin insert based protocol.
inent C3 deposition, were used as positive controls for the C3 staining (13). For Ig, IgG1, IgG2a, and HA in immune complex GN, fluorescence intensity (minimum 20 glomeruli per mouse) was assessed (0 to 3+). Further quantitative evaluation of the extent of glomerular antibody deposition in HA-induced immune complex GN was made by capturing images of at least 10 randomly selected glomeruli (high power) from each mouse and analyzing mean fluorescence intensity in each glomerular tuft by tracing each tuft, after removing background values (i.e., light emanating from stained section without tissue) for each slide (NIH Image). Values are expressed as arbitrary units of fluorescence per pixel. The distribution within glomeruli of mouse Ig and horse apoferritin in glomeruli was assessed (minimum 20 glomeruli per mouse) by means of the following scale for each individual glomerulus: 1, deposition confined almost exclusively to mesangial areas; 2, predominant mesangial deposition but some capillary loop deposition; 3, predominant capillary loop deposition but some mesangial deposition; and 4, deposition confined almost exclusively to capillary loops.

Urinary protein excretions were determined by the Bradford method on 24-h urine collections from mice before disease and from each mouse after the final 24 h of the experiment. Urinary protein excretion was expressed in two ways: by 24-h values, and as a urinary protein to creatinine ratio. The latter to compensate for potential variability in 24-h collections from mouse to mouse. Urinary creatinine concentrations were measured by the alkaline picric acid method with an autoanalyzer, serum creatinine concentrations at the end of experiments by an enzymatic creatininase assay, and creatinine clearance by 24-h urine volumes, with urine and serum creatinine concentrations.

Renal collagen content was assessed by determining total hydroxyproline (24). Samples were hydrolyzed in 6 N HCl by incubation at 110°C overnight. The hydrolysate was neutralized with 2.5 M NaOH. Hydrolysates in isopropanol were oxidized by chloramine T, then mixed with p-dimethylaminobenzaldehyde (25 min, 60°C) and the absorbance measured at 558 nm. Total collagen was calculated with the assumption that collagen contains 12.7% hydroxyproline by weight. Results were expressed as μg/mg kidney wet weight.

Assessment of Systemic Immune Responses

Induction and assessment of dermal DTH is described above. To assess splenocyte cytokine production, splenocytes from mice with anti-GBM GN were prepared and cultured for 72 h with 10 μg/ml normal sheep IgG. IFN-γ and IL-4 in culture supernatants were measured by ELISA as described previously (25). Monoclonal antibodies used were rat anti-mouse IFN-γ (RA-6A2; Pharmingen, San Diego, CA) and biotinylated rat anti-mouse IFN-γ (XMG1.2; Pharmingen) for IFN-γ, and rat anti-mouse IL-4 (11B11; ATCC) and biotinylated rat anti-mouse IL-4 (BDV6; DNAX) for IL-4.

Circulating levels of total mouse anti-SG Ig were measured by ELISA as described previously (26) on serum collected at the end of experiments from mice with anti-GBM GN, at dilutions of 1 in 100, 1 in 400, 1 in 1600, and 1 in 6400. Detecting antibodies used were horseradish peroxidase–conjugated sheep anti-mouse Ig (Amersham, Little Chalfont, UK; 1 in 2000). Mouse anti-HA Ig was assessed by using a modification of the method used for mouse anti-sheep globulin Ig, with plates coated with 100 μl of 50 μg/ml HA at dilutions of 1 in 100, 1 in 200, 1 in 400, 1 in 800, and 1 in 1600. For IgG1 assessments (serum dilution 1 in 100 both anti-SG and anti-HA), horseradish peroxidase–conjugated goat anti-mouse IgG1 antibodies (Southern Biotechnology, Birmingham, AL; 1 in 4000) were used. For measurements of IgG2a (serum dilution 1 in 1000 anti-SG, 1 in 100 anti-HA), 2% casein was used to block plates, rat anti-mouse IgG2a mAb (Pharmingen, clone R19-15) was used as the detecting antibody, and the reaction was amplified with 1 μg/ml ExtrAvidin (Sigma), mouse anti-avidin antibody (0.5 μg/ml, Sigma, clone WC19.10), and ExtrAvidin-peroxidase (Sigma, 1.1 μg/ml).

Results

Confirmation of the Disruption of the IL-13 Gene

Experimental IL-13+/+ mice carried only the wild-type allele of exon 1 of the IL-13 gene. IL-13−/− mice were documented as carrying only the disrupted IL-13 exon 1 gene, and IL-13+/− mice carried both wild-type and disrupted genes (Figure 1).

Endogenous IL-13 in Cell-Mediated Crescentic GN

Glomerular Crescents and Cell-Mediated Injury Are Not Regulated by IL-13. Ten days after challenge with sheep anti-mouse GBM globulin, sensitized IL-13+/+ mice (n = 7) developed severe diffuse proliferative and crescentic GN (Figures 2A and 3A). CD4+ cells, CD8+ cells, and macrophages were present in glomeruli. (Figure 3, B through D). Severe proteinuria and significant renal impairment were present at the end of the experiment (Figure 4). In IL-13+/+ mice (n = 3), crescent formation (38.7% ± 5.5% of glomeruli affected) and urinary protein excretion (10.9 ± 2.0 mg/24 h, protein:creatinine ratio 1.66 ± 0.35 mg/μmol) was similar to that found in IL-13+/+ mice (37.0% ± 6.1% of glomeruli affected, 16.9 ± 4.4 mg/24 h and 2.60 ± 0.52 mg/μmol protein:creatinine ratio). The absence of endogenous IL-13 in IL-13−/− mice (n = 9) did not alter cell-mediated components of this disease, namely the degree of glomerular crescent formation, or the accumulation of CD4+ cells, CD8+ cells, and macrophages in glomeruli (Figures 2B and 3).

Antigen-specific Humoral Responses Are Enhanced in the Absence of IL-13. However, humoral responses were enhanced by the absence of endogenous IL-13. Autologous antibody and C3 were detected in glomeruli by immunofluorescence (Table 1, Figure 2C) and assessed by semiquantitative

Figure 1. Confirmation of the presence of the disrupted IL-13 gene in IL-13−/− mice, the presence of only wild-type alleles in IL-13+/+ mice, and both wild-type and disrupted alleles in IL-13+/− mice by means of a PCR-based protocol. Separate sets of primers detected either the disrupted IL-13 allele (A) or the wild-type IL-13 allele (B). Lanes 1 to 3 show mice with only the wild-type allele of exon 1 of the murine IL-13 gene (IL-13+/+ mice); lanes 4 to 6 show mice with the disrupted allele (IL-13−/− mice); and lanes 7 to 9 demonstrate IL-13+/− mice with one copy of both the wild-type and the disrupted exon 1 of the IL-13 gene. Molecular markers are in 100-bp divisions.
scoring of fluorescence intensity and the end point positive titer of fluorescence, as previously described (19). In the absence of IL-13, there was increased autologous antibody in glomeruli of mice with GN (Table 1, Figure 2D), but C3 deposition was similar in IL-13+/+ and IL-13+/− mice. In serum of mice with GN, antigen-specific antibody titers were increased in the absence of endogenous IL-13 (Figure 5). Analysis of the serum antigen-specific IgG subclasses IgG1 and IgG2a showed an increase in the Th1-associated IgG2a subclass in IL-13+/− mice.

In Cell-Mediated GN, Functional Renal Injury Is Unchanged in the Absence of IL-13. Renal injury in this model is mediated predominantly by CD4+ T cells and macrophages (13,17). Despite enhancement of humoral responses in the absence of IL-13, urinary protein excretion and impairment of renal function (measured by the fall in creatinine clearance) was similar both in the presence and absence of IL-13 (Figure 4).

Systemic Cellular Immune Responses to Sheep Globulin in IL-13+/− Mice. Systemic immune responses to SG were assessed in the presence and absence of endogenous IL-13. Dermal DTH responses to SG were assessed by antigen challenge into footpads and ears of sensitized mice. No significant difference was found in DTH responses to SG (Table 2). Antigen stimulated splenocytes from IL-13−/− mice produced similar amounts of IFN-γ, but less IL-4 than in IL-13−/− intact animals.

Endogenous IL-13 in Humorally Mediated Renal Injury Increased Proteinuria in the Absence of IL-13. IL-13+/+ (n = 6), IL-13+/− (n = 6), and IL-13−/− (n = 6)
glomeruli (27). The deposition of immune complexes resulted with consequent immune complex formation and deposition in model results from humoral immune responses against HA mice were repeatedly immunized with HA. Renal injury in this model results from humoral immune responses against HA with consequent immune complex formation and deposition in glomeruli (27). The deposition of immune complexes resulted in mild glomerular injury and only mild proteinuria in IL-13+/+ mice and IL-13+/− mice (Figure 6, A and B; Figure 7). IL-13−/− mice developed increased proteinuria compared with IL-13+/+ mice (Figure 7). IL-13+/+ and IL-13+/+ mice had normal creatinine clearance, but the creatinine clearance of IL-13−/− mice was higher than that of IL-13+/+ mice. Total glomerular cell numbers and glomerular macrophage and neutrophil accumulation were similar in all three groups of mice with GN (Table 3).

Humoral Immune Responses Are Altered in the Absence of IL-13. Both Ig and HA were deposited in a granular fashion in mice immunized with HA (Figure 6, D through I). Although there was no marked increased in the degree of deposition of HA and mouse Ig in glomeruli (Figure 8, A and B), quantitative analysis of mouse Ig deposition in glomeruli showed an increase in IL-13−/− mice compared with IL-13+/+ mice that did not reach significance compared with IL-13+/+ mice (Figure 8C). The deposition of IgG1 (a Th2-associated subclass) in glomeruli was unchanged in the presence or absence of IL-13 (Figure 8D), but IgG2a (a Th1-associated subclass) deposition was increased in IL-13−/− mice (Figure 8E). In addition to these alterations in the composition of immune complexes in glomeruli, the distribution of immune complex deposition was different in IL-13−/− mice.

Table 1. Immunoglobulin and C3 in glomeruli of IL-13+/+ (n = 7) and IL-13−/− (n = 9) mice with cell-mediated crescentic glomerulonephritis

<table>
<thead>
<tr>
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<th>IL-13+/+</th>
<th>IL-13−/−</th>
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<tr>
<td>Mouse immunoglobulina</td>
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<tr>
<td>Intensity (0−3+)</td>
<td>1.1 ± 0.1</td>
<td>2.1 ± 0.2b</td>
</tr>
<tr>
<td>End point titer (log2)</td>
<td>10.9 ± 0.45</td>
<td>12.1 ± 0.28c</td>
</tr>
<tr>
<td>C3</td>
<td>1.3 ± 0.2</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td>End point titer (log2)</td>
<td>11.7 ± 0.3</td>
<td>11.9 ± 0.1</td>
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a Tissue sections were assessed by semiquantitative assessment of fluorescence intensity at a dilution of 1 in 100 and by the end point positive titer. Results are expressed as the mean ± SEM.
b P < 0.01, c P = 0.04 (unpaired t test).

Table 2. Systemic immune responses to sheep globulin in IL-13+/+ and IL-13−/− mice

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<tr>
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<th>IL-13+/+</th>
<th>IL-13−/−</th>
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<tr>
<td>DTHa (footpad, mm)</td>
<td>0.62 ± 0.11</td>
<td>0.39 ± 0.04b</td>
</tr>
<tr>
<td>DTH (ear swelling, mm)</td>
<td>0.56 ± 0.08</td>
<td>0.92 ± 0.21b</td>
</tr>
<tr>
<td>IFN-γc (pg/ml)</td>
<td>241 ± 92</td>
<td>337 ± 186</td>
</tr>
<tr>
<td>IL-4d (pg/ml)</td>
<td>272 ± 43</td>
<td>117 ± 33d</td>
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a Footpad and ear swelling DTH values (IL-13+/+ n = 7, IL-13−/− n = 5) are expressed as the difference between the footpad or ear injected with sheep globulin and that injected with the control antigen (horse immunoglobulin). Results are expressed as the mean ± SEM. DTH, delayed type hypersensitivity.
b Not significant, P > 0.1 (unpaired t test).
c Values for IFN-γ and IL-4 (IL-13+/+ n = 7, IL-13−/− n = 9) represent values obtained by ELISA of supernatants from antigen stimulated splenocytes from mice with anti-GBM GN (cultured at 4 × 10⁷/ml) after 72 h.
d P = 0.01 (unpaired t test).
compared with IL-13+/+ or IL-13+/− mice (Figure 6, D through I, and Figure 9). In IL-13+/+ mice both apoferritin and mouse Ig deposits were concentrated in mesangial regions as previously reported in this model (27). In IL-13+/− mice, although there were often deposits in mesangial regions, both antigen and antibody were present more prominently in and around capillary loops. Analysis of the overall pattern of Ig and HA deposition in mice in 20 or more individual glomeruli of all mice (a score of 1 being predominantly mesangial and a score of 4 being predominantly peripheral) confirmed this altered pattern of immune complex distribution (Figure 9). Complement (C3) was not detected in glomeruli of affected animals consistently above background staining seen in normal mice without GN (dilutions 1:25, 1:50, or 1:100).

Compared with IL-13+/+ mice, IL-13−/− mice had higher titers of anti-HA antibodies in the serum (Figure 10). In contrast to the findings in glomeruli IL-13+/+ mice developed similar titers to IL-13−/− mice. However, on analysis of IgG subclasses, elevated levels of both IgG1 and IgG2a were present in IL-13−/− mice compared with IL-13+/+ and IL-13+/− mice. IgG subclass levels in IL-13+/− mice were not elevated when compared with IL-13+/+ mice.

Endogenous IL-13 in Chronic Immune-Mediated GN.
Nonsensitized IL-13+/+ (n = 5) and IL-13−/− (n = 6) mice were injected with 16 mg of anti-GBM globulin and experiments terminated at 6 wk. After this more prolonged disease, the total collagen content of nephritic kidneys had increased by approximately 25% (normal mouse kidney 4.6 ± 0.2 μg collagen/mg tissue, IL-13+/+ mice 5.8 ± 0.2 μg collagen/mg tissue P < 0.001, unpaired t test). Histologic appearances of IL-13+/+ and IL-13−/− mice with GN were similar at this time point (data not shown). IL-13−/− mice were not protected from this increase in total collagen (6.5 ± 0.4 μg collagen/mg tissue). Renal function, assessed by serum creatinine values (IL-13+/+ mice 18 ± 2 μmol/L; IL-13−/− mice 19 ± 1 μmol/L) and urinary protein excretion (IL-13+/+ mice 4.5 ± 0.7 mg/24 h; IL-13−/− mice 4.0 ± 0.4 mg/24 h), were also similar in the two groups.

Discussion
The glomerulonephritides are a collection of diseases in which a variety of immune effectors can induce injury. There is increasing evidence that crescentic GN, the most severe and rapidly progressive form of GN, is a manifestation of a DTH-like responses targeted to the glomerulus (11,28). Many other forms of GN are predominantly or even exclusively mediated by antibody or immune complexes (20). Renal fibrosis is the final common pathway of most forms of renal injury (29).
Glomerular and interstitial fibrosis is a late but important event in immune renal injury characterized by accumulation of collagen in the kidneys, signifying impending permanent loss of renal function. The study presented here addresses the role of IL-13 in antigen-induced GN.

IL-13 is a cytokine classically produced by Th2 cells (2). It has known Th2-promoting effects and, at least in Th2-directed injury, profibrotic effects (1), perhaps the result of stimulating and activating TGF-β (30), a growth factor important in renal fibrosis. Less is known about its potential effects in negatively regulating Th1 responses in vivo. The study presented here demonstrates that endogenous IL-13 limits humoral responses, Th1 IgG subclass switching, and humorally mediated injury; that IL-13 does not play an important role in regulating the cell-mediated component of damaging Th1 responses; and that in a Th1-directed model, endogenous IL-13 does not promote renal collagen accumulation.

Table 3. Total glomerular cell number and leukocytes in glomeruli of IL-13+/+, IL-13+/−, and IL-13−/− mice (n = 6 each group) with apoferritin-induced immune complex glomerulonephritis

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<th>IL-13+/−</th>
<th>IL-13−/−</th>
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<tr>
<td>Glomerular cell number (c/gcs)</td>
<td>38.9 ± 0.2</td>
<td>35.6 ± 1.9</td>
<td>37.2 ± 0.7</td>
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<tr>
<td>Macrophages (c/gcs)</td>
<td>0.53 ± 0.09</td>
<td>0.53 ± 0.06</td>
<td>0.53 ± 0.07</td>
</tr>
<tr>
<td>Neutrophils (c/gcs)</td>
<td>0.36 ± 0.06</td>
<td>0.41 ± 0.06</td>
<td>0.27 ± 0.04</td>
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a No alterations in cellularity or glomerular leukocyte accumulation were observed.

b Results are expressed as the mean ± SEM. c/gcs, cells per glomerular cross section.

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Murine crescentic GN induced by planted an exogenous antigen (sheep globulin) in glomeruli of sensitized mice (autologous anti-GBM GN) is Th1 directed (13,14,19), effector

Figure 8. Intensity of immune complex deposition in mice with immune complex glomerulonephritis (GN) (n = 6 each group), assessed by the glomerular deposition of the disease-initiating antigen horse apoferritin (HA), mouse Ig, and IgG1 and IgG2a subclasses. There were trends to increased HA (A) and Ig (B) deposition when assessed semiquantitatively on a 0 to 3+ scale (ANOVA, P = 0.052 [A] and P = 0.06 [B]). Quantitation of Ig deposition by image capture and NIH image analysis of fluorescence from glomeruli (C) showed increased Ig deposition in IL-13−/− mice that reached statistical significance when compared with IL-13+/+ mice. Glomerular IgG1 deposition was unchanged (D), but there was increased IgG2a deposition (E) in IL-13−/− mice. * P < 0.05 versus IL-13+/+ mice; ** P < 0.01 versus IL-13+/+ mice, *** P < 0.0001 versus both IL-13−/− and IL-13+/− mice (ANOVA).

Figure 9. Semiquantitative analysis of the pattern of immune complex deposition (mouse Ig and horse apoferritin [HA], n = 6 each group) in glomeruli, assessed (at least 20 glomeruli) on a scale of 1 to 4, with 1 being deposition confined almost exclusively to mesangial areas and 4 being deposition confined almost exclusively to capillary loops, thus showing an alteration in the pattern of immune complex deposition in IL-13−/− mice toward capillary loops. * P < 0.01, ** P < 0.001 versus IL-13+/+ and IL-13+/− mice (ANOVA).
IL-13 would limit injury in experimental Th1-directed, DTH-like crescentic GN is relevant to disease pathogenesis and potentially treatment.

In autologous (T cell-mediated) anti-GBM GN, mice deficient in IL-13 showed no increase in crescent formation, accumulation of T cells or macrophages, and no increased functional injury. Dermal DTH was unchanged in the absence of IL-13, and splenocyte IFN-γ production was not increased. IL-4 production was reduced, consistent with at least some of the data on the phenotype of this mouse (5). However, in the absence of IL-13, SG-specific antibody levels were increased in serum and glomeruli in mice with anti-GBM GN. Although IgG1 levels were essentially unchanged, consistent with previous reports (3), isotype switching toward IgG2a did occur. These results demonstrate that unlike endogenous IL-4 and IL-10, which play important roles in limiting DTH responses and crescentic GN, IL-13 does not play a major role in limiting Th1 cell-mediated injury induced by exogenous antigens. Because this lesion is not humorally mediated, the increased humoral responses in IL-13−/− mice did not translate into increased functional injury.

Given these increased humoral responses in the absence of endogenous IL-13 in GN, with increased Th1 subclass switching in the absence of clear increases in cell-mediated immunity, the role of endogenous IL-13 was studied in a model of GN (anti-apoferritin immune complex GN) where autologous humoral responses are important. Increased serum antibody responses and switching to IgG2a were confirmed in this model, although there was some discordance between serum antigen–specific antibody levels measured by ELISA and the assessment of Ig in glomeruli by semiquantitative and quantitative methods. Increased functional renal injury, in the form of increased urinary protein excretion, was present in IL-13−/− mice. However, immune complexes that formed in the absence of IL-13 were situated both in mesangial areas and around glomerular capillary loops. The combination of the increased in glomerular immune complex deposition, the alterations in IgG2a deposition, and the disruption of the size/charge barrier of the GBM by immune complexes deposited in glomerular capillary wall is likely to explain the increased proteinuria seen in the absence of IL-13. There was no increase in cellular effectors of immune complex injury (macrophages and neutrophils in glomeruli), and significant glomerular C3 deposition was not observed. Renal function in IL-13−/− mice with this form of GN was significantly increased over normal and may reflect hyperfiltration associated with abnormal proteinuria.

IL-13 plays a role in fibrotic liver and lung disease (reviewed in (1)). The Th1-directed, autologous anti-GBM model was extended to 6 wk by use of nonsensitized mice. Renal fibrosis is a feature of most progressive nephropathies and is characterized by accumulation of collagen in glomeruli and the interstitium. By 6 wk in autologous anti-GBM GN, inflammatory injury is chronic and there is a significant increase in total renal collagen levels in genetically normal mice. However, we did not observe any further increase in renal collagen in IL-13−/− mice, nor did they at that stage have increased urinary protein excretion or renal impairment compared with IL-13+/+ mice. It is possible that a subtle increase in more chronic injury due to enhanced antibody responses in IL-13−/− mice might have masked a profibrotic effect of IL-13, resulting in no net increase in collagen content. In addition, the
degree of collagen accumulation and renal impairment in this model at the time point examined was not severe, and potential differences between *IL-13*+/+ and *IL-13*−/− mice might not be apparent at this stage. Previous studies have shown an important pathogenetic role for *IL-13* in fibrotic liver and lung injury mediated by Th2 responses (6,30). The data presented here suggests that this may not be generalizable to all inflammatory responses that result in fibrosis.

There is limited information regarding *IL-13* in renal disease. *IL-13* mRNA is present in acute anti-GBM GN induced in nonsensitized rats (31). *IL-13* is expressed by renal tubular cells in experimental sepsis (32) and mesangial cells in culture respond to *IL-13* (33). Much of the work on *IL-13* in renal disease relates to its possible role in minimal change disease, a form of GN characterized not by leukocyte infiltration or antibody deposition, but by alterations in the size/charge selectivity of the GBM leading to proteinuria. There is evidence that systemic overproduction of *IL-13* may be important in the pathogenesis of this disease (34,35), that could potentially lead to alterations in GBM size/charge selectivity. However, our studies have focused on the role of endogenous *IL-13* as a cytokine in the generation of nephritogenic adaptive (i.e., antigen driven) immune responses. We have found that endogenous *IL-13* limits humeral responses, suppresses production of damaging Th1-associated IgG subclasses, and affects immune complex deposition in the kidney. However, in contrast to two other important Th2 cytokines, *IL-4* and *IL-10*, *IL-13* does not regulate or limit nephritogenic Th1-directed, DTH-like cell-mediated immune responses that lead to experimental crescentic GN.

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